

09/26/98

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 4453/0A/EP-Ts	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/EP 97/05393	International filing date (day/month/year) 01/10/1997	(Earliest) Priority Date (day/month/year) 03/10/1996
Applicant BOEHRINGER MANNHEIM GMBH et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Certain claims were found unsearchable (see Box I).
2. Unity of invention is lacking (see Box II).
3. The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
 - filed with the international application.
 - furnished by the applicant separately from the international application,
 - but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
 - Transcribed by this Authority
4. With regard to the title,
 - the text is approved as submitted by the applicant.
 - the text has been established by this Authority to read as follows:
5. With regard to the abstract,
 - the text is approved as submitted by the applicant.
 - the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.
6. The figure of the drawings to be published with the abstract is:

Figure No. _____

 - as suggested by the applicant.
 - because the applicant failed to suggest a figure.
 - because this figure better characterizes the invention.

None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/05393A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/52 C12N9/12 C12N15/70 C12N1/21 C12P21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category ^o	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 701 000 A (NEW ENGLAND BIOLABS INC.) 13 March 1996	1-6,8, 12,14, 15,18-22
Y	see page 3, line 10 - line 38 see page 4, line 54 - page 5, line 44; example 21 ---	7
X	SOUTHWORTH, M.W. ET AL.: "Cloning of thermostable DNA polymerases from hyperthermophilic marine Archaea with emphasis on Thermococcus sp. 9°N-7 and mutations affecting 3'-5' exonuclease activity" PROC.NATL.ACAD.SCI.USA, vol. 93, no. 11, 28 May 1996, pages 5281-5285, XP000652319 see the whole document ---	1,3-6,8, 12,14, 15,18
Y	---	7
		-/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

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Date of the actual completion of the international search 7 January 1998	Date of mailing of the international search report 30.01.98
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Donath, C

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/05393

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92 03556 A (CETUS CORPORATION) 5 March 1992 see page 2, line 28 - page 3, line 16 see page 23, line 21 - line 37 ---	7
A	WO 92 09689 A (STRATAGENE) 11 June 1992 see page 11, line 26 - page 15, line 19 see page 19, line 1 - page 25, line 29; examples 3-5,9 ---	1-22
A	PERLER, F.B. ET AL.: "Thermostable DNA Polymerases" ADVANCES IN PROTEIN CHEMISTRY, RICHARDS, F.M. ET AL. (ED.), ACADEMIC PRESS LONDON, vol. 48, 1996, pages 377-435, XP000654858 see the whole document -----	1-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 97/05393

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP 701000 A	13-03-96	JP 8168376 A		02-07-96
WO 9203556 A	05-03-92	US 5374553 A		20-12-94
		AU 653747 B		13-10-94
		AU 8501491 A		17-03-92
		CA 2089495 A		14-02-92
		DE 544789 T		16-12-93
		EP 0544789 A		09-06-93
		JP 8298991 A		19-11-96
		JP 2584198 B		19-02-97
		JP 7147990 A		13-06-95
		JP 7108220 B		22-11-95
		JP 6500020 T		06-01-94
		US 5624833 A		29-04-97
		US 5420029 A		30-05-95
WO 9209689 A	11-06-92	US 5545552 A		13-08-96
		US 5489523 A		06-02-96
		AU 9165991 A		25-06-92
		IE 62855 B		08-03-95
		WO 9209200 A		11-06-92
		US 5492890 A		20-02-96

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

Date of mailing (day/month/year)
08 February 1999 (08.02.99)

From the INTERNATIONAL BUREAU

To:

BOEHRINGER MANNHEIM GMBH
Patent Department
D-68298 Mannheim
ALLEMAGNE

Applicant's or agent's file reference 4453/OA/EP-Ts	IMPORTANT NOTIFICATION
International application No. PCT/EP97/05393	International filing date (day/month/year) 01 October 1997 (01.10.97)

1. The following indications appeared on record concerning:

the applicant the inventor the agent the common representative

Name and Address BOEHRINGER MANNHEIM GMBH D-68298 Mannheim Germany	State of Nationality DE	State of Residence DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

the person the name the address the nationality the residence

Name and Address ROCHE DIAGNOSTICS GMBH D-68298 Mannheim Germany	State of Nationality DE	State of Residence DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:
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the receiving Office the designated Offices concerned
 the International Searching Authority the elected Offices concerned
 the International Preliminary Examining Authority other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Beate Giffo-Schmitt Telephone No.: (41-22) 338.83.38
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PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION
(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

United States Patent and Trademark
Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing: 09 April 1998 (09.04.98)	
International application No.: PCT/EP97/05393	Applicant's or agent's file reference: 4453/OA/EP-Ts
International filing date: 01 October 1997 (01.10.97)	Priority date: 03 October 1996 (03.10.96)
Applicant: ANKENBAUER, Waltraud et al	

1. The designated Office is hereby notified of its election made:

in the demand filed with the International preliminary Examining Authority on:
28 February 1998 (28.02.98)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer: J. Zahra Telephone No.: (41-22) 338.83.38
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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT PCT

REC'D 09 FEB 1999

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 4453/OA/WO-Ts	FOR FURTHER ACTION		See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
International application No. PCT/EP97/05393	International filing date (day/month/year) 01/10/1997	Priority date (day/month/year) 03/10/1996	
International Patent Classification (IPC) or national classification and IPC C12N15/52			
Applicant BOEHRINGER MANNHEIM GMBH et al.			

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

- I Basis of the report
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

Date of submission of the demand 28/02/1998	Date of completion of this report 03.02.99
Name and mailing address of the IPEA/ European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer Donath, C Telephone No. (+49-89) 2399-8710



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP97/05393

I. Basis of the report

1. This report has been drawn on the basis of (substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

Description, pages:

1-35 as originally filed

Claims, No.:

1-22 as received on 13/01/1999 with letter of 13/01/1999

Drawings, sheets:

1/16-16/16 as originally filed

2. The amendments have resulted in the cancellation of:

- the description, pages:
 - the claims, Nos.:
 - the drawings, sheets:

3. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP97/05393

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 1-22
No: Claims

Inventive step (IS) Yes: Claims 1-22
No: Claims

Industrial applicability (IA) Yes: Claims 1-22
No: Claims

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP97/05393

Ad section V.:

1. The following document is cited:

D1 EP-A-0 701 000

D2 WO-A-92/09689

2. The present International application refers to a thermostable DNA polymerase obtainable from *Thermococcus gorgonarius*, a DNA sequence encoding said DNA polymerase, a vector containing said DNA sequence, a microbial host transformed with said vector, a process for the preparation of said DNA polymerase and the use of said DNA polymerase in different processes for amplifying DNA, cDNA cloning, DNA sequencing and for DNA labelling.

In view of the documents cited in the International Search Report the subject-matter of claims 1-22 of the present International application has to be regarded as being new (Article 33(2) PCT).

3. The closest prior art to evaluate the inventiveness of claims 1 - 22 of the present International application is document D1.

D1 describes the cloning, characterization and preparation of a thermostable DNA polymerase from archebacterium *Thermococcus* sp. 9°N-7 (9°N-7). The 9°N-7 DNA polymerase has a molecular weight of about 90-95 kDa as measured by SDS-PAGE, possesses a very strong 3'- 5' proofreading exonuclease activity which is about 5 times that of the DNA polymerase obtainable from *T. litoralis*, and has an estimated half-life of about 5-6 hours at 95°C. The DNA sequence encoding the 9°N-7 DNA polymerase has been cloned and was over-expressed in *E. coli* under the control of a phage T7 promoter in an appropriate vector. The 3'-5' - exonuclease motif I of 9°N-7 DNA polymerase has been mutagenized resulting in a mutant having reduced but not completely eliminated the 3'-5' exonuclease activity to less than about 10% of the wild-type recombinant 9°N-7 DNA polymerase (see D1, p.3, I.10-38, p.4, I.54 - p.5, I.44, example 21).

In the prior art no indication has been given for a thermostable DNA polymerase possessing 3'- 5' -exonuclease activity and exhibiting at least a two-fold greater replication fidelity than DNA polymerase obtainable from *Pyrococcus furiosus*

INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/EP97/05393

(Pfu). From the prior art as illustrated by D2, concerning the purification, characterization and cloning of the DNA polymerase from *Pyrococcus furiosus*, it is known that Pfu DNA polymerase exhibits a very high fidelity in PCR studies in comparison to other known thermostable DNA polymerases. The DNA polymerase disclosed in D1, however, although having a very strong 3'- 5' - exonuclease activity, was shown to exhibit a tendency to degrade single stranded DNA (primer) and therefore, it cannot be successfully used in PCR.

Thus, an inventive step has to be acknowledged for claims 1 - 22 of the present International application (Article 33(3) PCT).

Ad section VIII.:

1. Claims 1-10, 12-22 lack clarity because, a protein or a DNA sequence - being a chemical product - has to be characterized by structural features, e.g. by its sequence, its molecular weight or as a product by process, but not merely by a result to be achieved or by its function.

The applicant is informed that the feature "obtainable from *Thermococcus gorgonarius*" has no limiting effect on the scope of the claims, as long as a thermostable DNA polymerase having the same biological properties than that of *Thermococcus gorgonarius* can be obtained from another microorganism. Moreover, the characterization of the claimed thermostable DNA polymerase **only** by the functional parameter "at least a two-fold greater replication fidelity than DNA polymerase obtainable from *Pyrococcus furiosus*" can only be allowed if it is unambiguously clear, that the applicant does not disguise a lack of novelty of the claims by the use of said parameter.

2. Claim 16 lacks clarity due to the wording of the claim. Not the known E.coli strain LE392pUBS520 has the deposition number DSM No.11328, but the E.coli strain LE392pUBS520 being transformed with the vector of claims 12 - 14.